Norfloxacin – a whole body physiologically based pharmacokinetic model

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Introduction
- The extent of tissue penetration, distribution and the size of the therapeutic window of antibiotics are among the most important issues confronting antibiotic therapies.
- Whole body physiologically based pharmacokinetic (WBPBPK) models are increasingly used to predict the pharmacokinetic behaviour of drugs due to its improved accuracy and flexibility over conventional PK models. It has also been used extensively in the extrapolation of pharmacokinetics to man.
- Norfloxacin is a fluoroquinolone antibiotic used mainly in genitourinary infections and it is known to be widely distributed. Nonlinear pharmacokinetics have been reported and this is investigated in this study.

Aims
- To develop a WBPBPK model to study norfloxacin kinetics in rats using tissue concentration and arterial blood data.
- To compare 4 methods of Kp estimation.

Methods & Assumptions
- The model was developed using volumes and blood flows taken from Brown et al (1) for rats weighing 320g.
- Norfloxacin was administered in 3 dose groups (25, 50 and 100 mg/kg) via intravenous bolus to male Sprague-Dawley rats (n = 15 per dose group), serial blood samples were taken (4 times points per animal) and tissues (fig. 1) were excised (30, 60, 180, 300 and 420 minutes; n = 3 per time point). Plasma and tissues were quantified using HPLC with fluorescence detection.
- Total plasma clearance was estimated using NONMEM (FO, proportional residual error) based on the arterial plasma concentrations using a 2-compartmental model with IV bolus input. Since the renal clearance was not experimentally measured, 15% of each plasma clearance value was taken to be the renal clearance for each dose and this was fixed in the model (3).
- Tissue affinities (tissue-blood partition coefficient, Kp) were calculated using 4 methods (4):
  1. Steady state measurement (Kpss)
  2. Area ratio method (KpAUC)
  3. Open-loop method (Kpclosed)
  4. Closed-loop method (Kploop)
- Steady state (SS) measurement: SS was obtained via an IV bolus dose and a constant-rate IV infusion to reach a concentration of 30 µM (0.01 mg/mL). Kpss was calculated as follows:
  \[ Kp_{SS} = \frac{C_{SS}}{C_{ss}(1-E)} \]
  where \( C_{SS} \) is the SS concentration in the tissue, \( C_{ss} \) is the SS venous plasma concentration, \( C_{ss} \) is the SS concentration in the arterial plasma and E is the extraction ratio for (eliminating tissues only).
- Area ratio method: this involved using Kinetica 4.1.1 to compute the area under the curve (AUC) for each compartment from zero to infinity by interpolation using the log-linear trapezoidal method before calculating the Kpss as follows:
  \[ Kp_{AUC} = \frac{\int C_{ss} \, dt}{\int C_{ss} \, dt} \]
  Eliminating tissues
  \[ Kp_{AUC} = \frac{AUC_{tiss}}{AUC_{art}(1-E)} \]
- Open-loop method: a 2-compartmental IV model was used to compute a forcing input function for each dose. Measured concentrations of the compartments were then fitted in Matlab 6.1 to the WBPBPK model using nonlinear least-squares regression. Only compartments receiving input from arterial blood were included (exclude lung). Gut clearance was estimated at this stage.
- Closed-loop method: all compartments were fitted to the WBPBPK model using nonlinear least squares regression, as in the open-loop method and including the lung.

Results
- Clearance values obtained were in the same range as published values (5) but show no downward trend with increasing dose. Chi square test indicates that the differences between the clearance values for each dose are statistically significant (P < 0.05).
- The SS volume of distribution (Vss) values (range: 3505 - 3602) are comparable to published values (range: 2991 – 3533) but do not indicate nonlinearity.
- Kp values compare well across doses and methods, although discrepancies were observed for the kidney and liver. These could be due to clearance issues: the fraction unbound (fu) and CLtotal were not experimentally determined and literature values were used instead.

Figure 1. Whole body physiological model for norfloxacin

Legend:
VEN: Venous; ART: Arterial; L.U: Lung; B.P: Brain; H.T: Heart; L: Liver; ST: Stomach; GUT: Duodenum, jejunum & ileum; K: Kidney; MU: Muscle; SK: Skin; AD: Adipose; REST: Rest of the body; CL: Clearance

Figure 2. Comparison of clearance values with published data

Figure 3. Comparison of KpSS, KpAUC and Kploop vs Kpclosed

Figure 4. (a) Comparison of KpAUC with Kpss, and (b) Kpclosed vs Kpss

Figure 5. Concentration-time graphs for lung, heart, brain, adipose, venous and kidney.

Conclusion
- Kps from the 4 different calculation methods compare well against each other in most compartments.
- Nonlinearity could not be proven from this study, although the clearances are in the same range as published values. This could be due to high inter-animal variability.
- The closed-loop optimisations produce the best fits with measured data.
- The tissues in this model appear to be perfusion rate limited.
- The closed-loop method produced the lowest RMSE values for data-fitting, suggesting that it is the best method for Kp estimation in this study.

References
2. This data was made available by M. Chenel, who carried out these experiments as part of her PhD at the University of Poitiers, France.

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